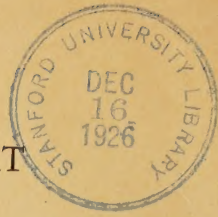


FREEZING AND SURVIVAL OF INSECTS AT
LOW TEMPERATURE



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EXTREMES of temperature are important factors in the ecology of all animals. Low temperatures affect the geographical distribution of insects, their cold resistance, rate of metabolism, behavior, and in some cases even their color and structure. This paper is the result of studies of the effect of low temperatures on cold resistance in insects with especial reference to temperatures below the freezing point of water.

Three ecological groups of insects were chosen for study: (1) the oak borers living close to the outer surface of the bark, normally exposed to extremes of low temperature; (2) aquatic insects living in lakes, never exposed to temperatures lower than 0°C.; (3) insects infesting stored products.

The necessity for the endurance of low temperature is a fundamental one which must be met by all organisms living in Northern climates. The literature on the biological effects of low temperatures is enormous. Literature relating to both plants and animals is cited in this paper for the reason that plant physiology has been advanced to a farther stage than insect physiology and offers many suggestions to workers in the animal field.

I. LITERATURE ON THE EFFECTS OF LOW TEMPERATURE ON PLANTS

There is an extensive and scattered literature on the freezing and hardening

in plants and on allied physiological effects of cold. It is interesting to discover that very early in the history of plant physiology, observations took on a quantitative aspect. Workers in this field of plant physiology include some of the foremost plant physiologists, as for example, Sachs and Pfeffer.

In plants, death from freezing has been considered to be due to the following causes: (1) mechanical injury from ice crystal formation, (2) water loss either direct or indirect through permeability changes in the cell membrane, (3) precipitation of proteins or other irreversible chemical effects. The order given in general follows the historic development of the study of cold effects on plants.

Duhamel and Buffon (1732) held that death from freezing was due to the rupture of tissue from ice crystals formed in freezing. Morren (1852) found no histological evidence in favor of this theory. Göppert (1829) found that ice formation took place in both cells and cellular spaces. Sachs (1860) discovered that in the process of freezing water was withdrawn from the cells to freeze for the most part in the intercellular spaces. From another source evidence was obtained against the cell-rupture theory when Nägeli (1860) calculated the expansion of all the water in a cell at freezing and found that the total expansion was insufficient to cause rupture. However, disproving that cell rupture is the cause

of death from low temperatures is not the equivalent of finding the cause of death from low temperatures, nor does the negative result obtained throw much light on the subject of death from this cause.

Water loss both direct and indirect was the next explanation for injury and death at low temperatures. Water loss from frozen tissue on thawing was observed by Göppert (1829). Prilleux (1869) discovered that water was exuded from the cells in the freezing process. Sachs (1860) noted that water exuded from the cells was "reabsorbed" or was lost by evaporation. Injury from freezing was thought to be due essentially to a dehydration. Another effect of low temperature analogous to the effect from freezing was dehydration at low temperatures above the freezing point. Water intake and water transportation at low temperatures is not rapid enough to balance transpiration. The plant exposed to such temperatures may wilt and finally die from the effects of low temperature without ever being frozen. Sachs (1860) points out these two dehydration effects of low temperature.

As an outgrowth of the more simple theory of direct water loss comes the theory of injury from water loss due to alteration of the permeability of the cell membrane at the time of freezing. Loss of water according to this view is not the primary cause of death from freezing, but permeability changes are. Maximow (1912) worked on sections of red cabbage and *Tradescantia*. He came to the conclusion that the part of the plant that was injured by low temperature was the plasma membrane. The immersion of sections of plant tissue in various solutions influenced the freezing point. Substances that enabled the cells to withstand a lower temperature than normally were called "Schützmittel" or protective sub-

stances. The degree of protection afforded by different substances was proportional to the eutectic point when the molecular concentrations were the same. Chandler (1913) came to the conclusion that the cause of death from freezing was due to mechanical injury of the plasma membrane. Death from freezing was limited to one condition, namely, the condition of brown wilted tissue resulting from exposure to freezing temperatures and subsequent thawing. Poor metabolism due to low temperature was not studied in Chandler's paper.

The third theory considers protein precipitation in the frozen cells accountable for death from freezing. The first man to elaborate this theory was Gorke (1906). His evidence was derived from plant cells and from solutions of proteins. The degree of hardiness in plants was correlated with the ease or difficulty with which their proteins were precipitated. In non-hardy begonia leaves protein precipitation took place at $-3^{\circ}\text{C}.$, in rye at $-15^{\circ}\text{C}.$, and in pine needles at $-40^{\circ}\text{C}.$ Schaffnit (1910) found that rye protein from plants grown under greenhouse conditions was more easily precipitated than rye protein from plants grown under outdoor conditions. Schaffnit's work will be taken up again when hardiness is discussed. Harvey (1918) found that a correlation existed between protein precipitation and hardiness. The influence of increased sugar content noted by Lidforss (1907), by Schaffnit (1910), and by later workers may be primarily as a protection to the proteins against precipitation, although such solutes would also lower the freezing point. Harvey (1919) laid emphasis on the relation of epidermal coverings to undercooling. He considered that concentration of cell solutes played a minor rôle in the determination of cold resistance in plants, the

principal effect coming from the precipitation of protein under the changed conditions produced by ice formation.

The development of hardiness in plants and the correlation of physical condition of cell colloids and of osmotic concentration of solutes with hardiness, is at present one of the extensively cultivated fields of plant physiology. By hardiness is meant the resistance of plants to low temperatures. Harvey has defined this term as the ability of a plant to survive ice formation within the tissues. The present writer uses the more general definition given first on account of the fact that the term hardiness has been used in the broader sense in much plant literature, and also for the reason that cold resistance in animals has not been studied sufficiently for us to know definitely the underlying cell changes that are responsible for increased resistance to cold.

Plant hardiness has long been of interest in horticulture and agriculture. Efforts to produce frost hardy plants are some of the new developments arising from the application of plant physiology and genetics. Factors that make for plant hardiness were studied in detail by Gorke (1906). Schaffnit (1910) found that cold hardiness in rye was increased by exposure of rye to low temperatures during growth. Lidforss (1907) noted that the sugar content of pine needles increased with the oncoming of winter. Maximow (1912) found that he could induce hardiness in plant tissues by soaking them in solutions of non-toxic substances. Among the substances used were glycerine, sucrose, mannite, and various nitrate salts. Rosa (1921) comes to the conclusion that any process which will bring about a material decrease in the rate of growth will result in an increase in hardiness. Decreasing the water content, increasing the amount of hydro-

phylic colloids present or increasing the water holding capacity of these colloids will result in an increase of hardiness, according to this investigator. Harvey (1918) studied the development of hardiness in plants and comes to the following conclusion.

The principal effect of the hardening process for cabbages is a change in the constituents of the protoplasm which prevents their precipitation as a result of the physical changes incident upon freezing. The proteins are changed to forms which are less easily precipitated. This is indicated by an increase in the amino-acid content of the cabbage plants on hardening.

. . . . The effects of desiccation, freezing, and plasmolysis are considered to be similar in that all these processes cause changes in the hydrogen-ion and salt concentration.

Studies on the concentration of the sap and its relation to hardiness have been made by Gortner and Harris (1914). Tropical plants have a lower osmotic concentration and a lower pressure than do plants of temperate zones. Newton (1924) worked on the relation of the concentration of cell sap to the winter killing of different varieties of wheat. The ability of the cell colloids to adsorb water was taken as a measure of the cold resistance of winter wheat. The process of hardening in this case is thought to be the process of water adsorption and water retention by the hydrophilic colloids.

The relation of water content to winter hardiness was stressed by Johnson (1919, 1921, and 1923). He used water content as an index to the hardiness of peach buds. Newton (1924) found that water content of winter wheat was a partial index to hardiness.

There is another aspect to the subject of freezing that was brought out by Müller-Thurgau (1880). He distinguished between freezing and death from freezing. He also contributed to the theory of

freezing and made valuable observations on low temperatures of trees in winter.

Göppert (1839) held that rapid thawing would cause death after freezing but slow thawing would enable plants to survive. He cited examples of saving plants by thawing them in cold water. Müller-Thurgau (1880) compared the rate of thawing in air and in water of the same temperature and found that the rate of thawing in water was greater than the rate in air. A wide series of plants were tested for injury after slow thawing and after rapid thawing. Some plants showed typical spots of frost injury but were not killed, others showed no injury, and still others were killed outright. Müller-Thurgau (1880) concluded that rate of thawing was not closely correlated to survival after freezing, and that plants that would be killed by rapid thawing could not be saved by slow thawing.

Müller-Thurgau (1880) figures ice crystals in his first paper and also "Eisdrüsen" or ice glands—centers of crystallization. He also gives a method for determining the amount of water formed into ice. He concludes that there can be no freezing to death without first ice formation. Death does not precede freezing but follows it. He also gives extended figures on the winter temperatures of the north and south sides of trees, and shows the alternate freezing and thawing to which trees are subjected. This last phase of his work is important in the present considerations for the oak borers are exposed to the same conditions as the trees which they inhabit.

Müller-Thurgau (1880, 1886) found that plant sap froze at higher temperatures than the whole plant. He explains this higher freezing point on the basis of capillarity and structure of protoplasm. The intact plant is able to hold its water and prevent part of it from freezing. Alto-

gether these papers of Müller-Thurgau form one of the most important contributions to plant freezing and hardening. He also made some general observations on freezing in animals. On account of the importance of the study of tree temperatures in the study of the oak borer group, the following work is quoted. Emerson (1889) studied the temperatures of tree trunks in Nebraska. Squires (1894) and later Harvey (1923) studied the temperatures of trees in Minnesota. Graham (1920, 1922), made a study of tree temperatures with special reference to the maximum temperature endured by insects living under the bark. The influence of the color of the bark on the temperature of the cambial layer was stressed by both Graham and Harvey. Black bark has a higher temperature at a given air temperature than white bark. In winter, according to Harvey, there may be as much as 25°C. difference between the temperature of the north and of the south sides of trees. Alternate freezing and thawing of a living cambial layer is produced by alternation of sunlight and shadow when a cloud passes.

II. LITERATURE ON THE EFFECTS OF LOW TEMPERATURE ON ANIMALS WITH SPECIAL REFERENCE TO INSECTS

The progress of low temperature studies on insects has not been as rapid, nor has the quantitative aspect been emphasized as much as has been the case in plant physiology. The large economic losses from tender plants and the consequent desire to develop hardy varieties have had no counterpart in applied entomology, with the exception of some work concerning the insecticidal effects of low temperature over given periods of time.

The problem of hardiness in insects is essentially a part of the problem of hibernation. Animals that live in cold cli-

mates must adapt themselves to the conditions as they are, or avoid these conditions by migration to warmer or better protected places. This paper deals with animals that are so situated that they cannot avoid the low temperatures of winter but must take the temperature just as it is.

Allied to the problem of hibernation is the question of heat regulation which plays an important part especially in the hibernation of mammals. Rasmussen (1916) gives a summary of the different theories of hibernation. During hibernation homoiothermal animals become poikilothermic. In this latter state they are able to endure cold and starvation which under other conditions would be fatal. The hibernation of mammals may throw some light on the allied problem, the hibernation of insects. Low temperature, high concentration of carbon dioxide, and periodicity, were the chief factors proposed as causes of hibernation. An incompletely homoiothermic mechanism was associated with hibernation. The influence of low temperature on the production of a state of lethargy holds for insects as well as other animals. Periodicity has been emphasized by many workers. The question of a heat regulating mechanism is not fully settled, but there is some evidence from the work of Newport (1837), Bachmetjew (1901), and Pirsch (1923) that insects do have a slight control of their bodily temperatures under different environmental conditions.

The earliest literature on insect hibernation is gleaned from collectors' notes and simple observation that insects are found at low temperatures or do pass the winter out-of-doors. These notes will be passed over, as they do not contribute vitally to the subject in hand.

Aristotle knew that insects hibernated, but did not seek an explanation for this phenomenon.

One of the most striking characteristics about the state of hibernation is its periodicity. Even the name suggests this characteristic. Kirby and Spence (1818) view the phenomenon of hibernation as periodic, although this is not explicitly stated. The action of cold it is pointed out is not sufficient to account for the state of hibernation. Insects in hibernation, it is noted, are able to withstand extremes of low temperature, fatal to them in the active state. The statement is also made that insects frozen as solid as a block of ice can live after being thawed out, but that some insects die from the effect of cold before the freezing point is reached. Hibernation is looked upon as a provision against starvation at a time when the food of insects, whether herbivorous or carnivorous is no longer obtainable.

Baumberger (1917) makes much of the factor of periodicity. Hibernation, according to this author, is essentially a rhythmic phenomenon. It does not occur in tropical insects and is not well fixed in animals more or less protected from winter temperatures. The most fixed and the most periodic hibernation periods occur in animals exposed to winter temperatures habitually.

The meaning of the state of hibernation especially in regard to change or lack of feeding in this state was observed experimentally by Réaumur (1740) and by Huber (1792) in bees. Bees were observed to be active inside their cluster throughout the winter. Réaumur observed that less food was eaten by a hive kept in the cold than one kept in a warm place. Clustering, according to this author, was a manifestation of torpidity. Huber considered that the winter cluster was not the result of torpidity but rather a means of avoiding torpidity by keeping the temperature high.

The state of lethargy in caterpillars was observed by Vaudoner (1827). He noted especially its essential periodicity and observed that this state was not due entirely to lack of food or to low temperatures. Vaudoner's work has been largely overlooked by later biologists. Scudder (1889) found that some caterpillars went into a state of lethargy or premature hibernation at midsummer. Others continued feeding till the onset of severe weather. Between the state of hibernation and activity were shorter and less completely passive periods in the life of some caterpillars. The idea of hibernation as a comparative state rather than an absolute state is here expressed though not explicitly. This idea will reappear again when the subject of cold resistance is taken up. Hibernation of partial broods is considered by Scudder as a means for preserving the species. Thus if part of a group of feeding caterpillars go into a state of lethargy at one time and part at another, the chances for the survival of the species is better.

Associated with hibernation are other low temperature effects such as the freezing point of insects under different conditions, undercooling, hardiness, and lowered rate of metabolism. The rate of metabolism will not be discussed in this paper except in so far as it bears directly on the general problem of hardiness.

The observations of cold resistance in insects are scattering and often indefinite. A certain insect seen on a cold day, or revived after being exposed to winter temperatures, is a common type of observation. However, not all the observations are of this indefinite kind, for Réaumur, who was interested in temperature, was making measurements of the temperatures borne by some little larvae found in wood. Réaumur (1736) placed

small larvae in a freezing mixture of marine salt and water. The larvae were placed in a glass tube and the temperature of the freezing mixture taken. Larvae were able to withstand -8° or -9° Réaumur without perishing. These experiments were performed in the years 1708 and 1709 but were published in his monumental work *Mémoires pour servir à l'Histoire des Insectes*. In this work he also quotes an experiment of Lister who found that insects frozen so hard that they sounded like stones when dropped into a glass survived. This experiment has been quoted many times and has entered into current thought regarding the subject of survival from freezing. The fact of becoming stiff, even hard, may not be a proof that the insect is actually frozen, although workers today as well as in the 18th century, assume the two states to be identical.

The taking of individual temperatures of insects was first accomplished by means of small mercury thermometers. Davy (1826), Rengger (1817), Regnault (1819), and Hausmann (1803) were pioneers in this work. Newport (1837) conducted extensive studies on temperatures of insects of several species and representing four orders. These workers laid the foundation for later work on insect temperature and cold resistance.

By far the most comprehensive work dealing with the subject of insect temperature up to the present time is the work of Bachmetjew (1901). He summarized the work of his predecessors, performed numerous experiments and formulated conclusions regarding insect temperatures and cold resistance in insects. He used the thermocouple to measure the temperatures of insects and to record freezing points. He emphasized the time rate of cooling or the "Abkühlings geschwindigkeits" as influencing the vital tempera-

ture minimum, or the lowest temperature at which insects could survive. He also correlated the amount of "sap" present with the freezing point. This term is not quite equivalent to moisture for it represents the difference in weight between a normal insect and a desiccated one. That the weight loss may not be entirely due to water was recognized by Bachmetjew (1899). The size and structure of the cell contents was also recognized by Bachmetjew as correlated with the freezing point. There is one conclusion that Bachmetjew makes with regard to his vital temperature minimum that is difficult to understand. The reaching of the undercooling point for the second time, according to this author, determines death. The undercooling point rather than the freezing point is considered as the critical point. With regard to death occurring at the time of reaching this undercooling point the second time, the data of this author admit of more than one interpretation. About 27 per cent of his results disagree with his conclusion.

Bachmetjew's contributions to the subject of insect freezing can be summed up as follows: (1) the time rate of cooling determines the undercooling point and hence the critical point; the more rapid the cooling the higher the undercooling point; (2) the per cent of "sap" is inversely proportional to the freezing point; (3) the point of death is reached when the insect reaches the undercooling temperature a second time. The lowest undercooling point that was obtained by this author was -15.7°R .

Gueylard and Portier (1916) found that the survival of *Cossus* and *Carpocapsa* on exposure to low temperatures depended upon the season of the year. In winter these larvae were able to survive -20°C . but were killed by that temperature in the spring. In a later paper Duval and

Portier (1921) make further observations on freezing points and emphasize further their periodicity. Another contribution made by these authors is the concept of a secondary freezing point. The first freezing point represents the freezing of body fluid while a lower freezing point of the same tissue represents the freezing of the cell substance itself.

Knight (1922) was able to demonstrate a marked periodicity in the freezing point of the Pentatomid, *Perillus bioculatus*. In the fall this bug gained hardiness and in the spring lost it. Knight considered that the freezing point as demonstrated by the thermocouple method was colloidal in nature. Repeated freezing of the same bug brought the freezing and undercooling points nearer and nearer together, with each successive freezing. This action parallels the setting and melting of gelatine and other colloids where the past history influences present behavior.

Pirsch (1923) in a study of the individual temperatures of honey bees found the freezing point was about -1°C . He tried three bees only as his paper dealt chiefly with other phases of the problem of insect temperatures.

Carter (1925) studied the freezing and undercooling points of the bean weevil *Mylabris obtectus*. He found that the insect when uninjured by piercing with the thermocouple was able to withstand lower temperatures than a pierced insect. He found no correlation between the time rate of cooling and the freezing or the undercooling points. The weevils showed no periodicity during the time studied. Representatives of the stored-products pests that would not be expected to show hardening were chosen for his work in order to simplify the problem.

The relation of water content to the freezing point, cold endurance, and hiber-

nation has been suggested by several authors but definitely worked out by comparatively few. Bachmetjew (1899) emphasized the relation between "sap content" and freezing and undercooling points. The work of Tower (1906, 1910) while open to question in some respects is, however, suggestive. The low water content of hibernating forms is emphasized. Breitenbecher (1910) associated with Tower found that he could bring potato beetles out of hibernation by watering the soil in which they were dormant. Bodine (1921, 1923) has found that hibernating grasshoppers possess a low rate of respiration accompanied by a low moisture content. With the breakup of hibernation the water relations are readjusted to a higher level. Hibernation is essentially a rejuvenating process from the standpoint of basal metabolism. Fink (1925) states that hibernating animals have a low respiratory quotient. The breaking up of hibernation in the codling moth by repeated soakings of the hibernating individuals was reported by Townsend (1924) at the fifth Washington Meeting of the American Association for the Advancement of Science.

Bottazzi and Bergami (1924) reported an irreversible precipitation occurring in ox blood serum when it was cooled to -25°C . They were studying the action of low temperatures upon fluid colloidal systems. Perhaps the most comprehensive work on the relation of water to metabolism is that of Babcock (1912). He studied metabolic water in both plants and animals, and states that during hibernation metabolic water is the source from which animals draw their necessary supplies. The amount of water in hibernating animals is lower than that in non-hibernating animals.

The measurement of the cold resistance

of insects has been dependent upon the measurement of temperature itself and upon the applications of methods of temperature measurement to insect material. The invention of the thermometer in 1714 by Fahrenheit, its perfection by Réaumur, together with the interest in measuring temperatures at that period furthered the study of insect physiology. But there is another method of measuring temperatures, namely the thermo-electric method. Volta in 1801 found that an electric current was set up at the junction of two unlike metals. In 1822 Seebeck, using the newly invented instrument, the galvanometer, measured the E.M.F. set up at junctions of unlike substances and correlated these measurements with temperature change. As early as 1831, Nobili and Melloni were recording the temperatures of insects and other small animals by means of the thermocouple.

III. ORIGINAL EXPERIMENTAL RESULTS

The work of the present writer has dealt with the ability of insects to survive temperatures lower than 0°C . The cold resistance was measured by the freezing and by the undercooling points. By freezing is meant actual crystallization of a given body fluid or tissue accompanied by the giving off of heat. By the undercooling point is meant the limiting temperature below the freezing point of a fluid, to which it can be cooled without freezing.

With the thermo-electric method the freezing and the undercooling points of three groups of insects were taken throughout their normal annual cycle. These ecological groups were, (1) the oak borers, normally exposed to temperature extremes; (2) the aquatic insects never exposed to a temperature lower than 0°C ., and (3) stored-products pests, represent-

ing, supposedly, a tropical or sub-tropical group.

With the oak borers the freezing and the undercooling points were periodic and varied with the seasons. The lowest freezing and undercooling occurred in winter; the highest in summer; with fall and spring determinations intermediate. A marked correlation between the environmental temperature and the insect freezing and undercooling was formed. The species used in this study were larvae of *Synchroa punctata* Neum., *Dendroides canadensis* Lec., *Elatér* sp., *Melanotus* sp., *Chrysobothris femorata*, *Romaleum rufulum* Hald., *Elaphidion mucronotatum* Fab. *Lep-tura nitens* Forst., *Arhopalus fulminans* Fab., *Xylotrechus colunus*, Fab., *Graphi-surus fasciatus* Deg., and the adult of *Nyctobates fasciatus* Deg.

A series of freezing and undercooling points of aquatic insects was also made. The genera used were *Enallagma*, *Ischnura*, *Gomphus*, and *Sympetrum* among the Odonata; *Leptocella*, *Phryganea*, and *Neuronia* among the Trichoptera; *Buena*, *Belostoma* and *Notonecta* among the Hemiptera; *Halipus*, *Cybister*, *Coptotomas* and *Dytiscus* among the Coleoptera. Nymphs of the Odonata, larvae of Trichoptera, adults of the Hemiptera and adults of the Coleoptera were used. Determinations were made in February, May, July and September. There appeared to be no periodicity in these insects. There also seemed to be no significant difference between different species, different orders or different stages of development. The mean undercooling of all aquatic insects was 1.52 ± 0.3 , the mean freezing 0.57 ± 0.03 .

The insects infesting stored products exhibited no apparent periodicity but were more variable in both undercooling and freezing than the aquatic group. Three species, *Tribolium confusum*, Duval,

Sitophilus granarius Linn., and *Pyralis farinalis* Linn. were studied with the following results:

INSECT	STAGE	NUMBER DETERMINED	MAXIMUM	MINIMUM	AVERAGE
Undercooling					
<i>Tribolium confusum</i> Duval.....	Adult	200	-6	-1.5	-4.5
<i>Tribolium confusum</i> Duval.....	Larvae	200	-8	-5	-5.6
<i>Sitophilus granarius</i> Linn.....	Adult	200	-14	-2.2	-6
<i>Pyralis farinalis</i> Linn.....	Larvae	500	-7	-4	-6
Freezing					
<i>T. confusum</i>	Adult	200	-4.6	-0.5	-2
<i>T. confusum</i>	Larvae	200	-8	-7.1	-3.6
<i>S. granarius</i> Linn...	Adult	200	-8	-1.4	-2.4
<i>P. farinalis</i>	Larvae	500	-7	-4	-6

The mode of undercooling with *Sitophilus granarius* was -4, of freezing -2. Thus both the mode of undercooling and of freezing differ from the mean. The determinations on this species indicate a highly variable population as regards cold resistance.

Although the oak borer group appears essentially periodic as regards resistance to low temperatures it is possible to alter this character by exposure to suitable temperatures and humidities. Exposure of insects to low temperatures in the summer will start the hardening process. Similarly exposure to high temperatures in the winter time will cause loss of hardiness. The results of these experiments would indicate that the freezing point and undercooling is not itself essentially periodic but is a response to a temperature condition that is periodic. Thus

in northern climates the succession of seasons is essentially periodic.

Cold hardiness can be produced experimentally (1) by exposure to low temperature or (2) by dehydration.

Conditions associated with low freezing and undercooling temperatures were studied. The most pronounced feature was found to be the low moisture content. The insects in fully hardened condition have a low moisture content, in non-hardy condition a high moisture content. Periodicity was exhibited in the moisture content. That of *Synchra Punctata* varied from 31.1 per cent in February to 54 per cent in August. With *Dendroides* the variation in moisture content was more marked, from 57.4 per cent to 73.5 per cent. Larvae were baked for four hours at 50°C.

Some experiments were tried in order to determine the nature of freezing. Repeated or multiple freezings were taken on the same insect or tissue. These freezings and undercoolings so taken remained constant and exhibited no hysteresis. Samples of blood taken from the aortae showed definite crystals. Transparent larvae also were seen to have crystals within at the time the freezing point was recorded. The process of freezing in this group was interpreted as crystalloidal. The first or primary freezing point is the freezing point of the blood.

It was found that when the oak borers were in winter condition they could survive freezing. However, on lowering the temperature still further a second undercooling point was found and a second freezing. This secondary freezing point was always fatal. For the oak borer group it was near -40°C. The tissue freezing was not definitely isolated but experiments pointed to the possibility of the tissue being either fat within the cells or nervous tissue.

The problem of survival of insects at

low temperatures was studied in considerable detail with special reference to temperatures below 0°C. It was found (1) that insects not fully hardened were killed when the primary freezing point was reached; (2) in fully hardened insects the primary freezing point was not fatal but there occurred a secondary freezing point that was. Experiments were run to determine the relationship between the freezing point and survival of insects when exposed to low temperatures for as long as twelve hours. It was found that insects with high freezing points were never able to withstand long exposure to low temperatures. However, insects with low freezing points could be killed by long exposure when a short exposure would not be fatal.

With insects not in fully hardened condition the absolute minimum fatal temperature or the lowest at which insects can survive can be defined as the undercooling point. The "vital temperature minimum" of Bachmetjew thus is the absolute minimum fatal temperature for the oak borers throughout the summer, early fall and late spring. During the remainder of the annual cycle, the limit of undercooling is no longer the absolute minimum fatal temperature but the secondary freezing point or some point lower than the primary undercooling must be sought. The secondary freezing point represents the freezing of some tissue other than the blood. One experiment was tried to obtain the freezing point of the nervous tissue. The central nervous systems of fifty larvae were dissected out, placed in a small tube and frozen. The undercooling point recorded was -48°C., the freezing point -45°C.

IV. SUMMARY

Due in part to demand for cold hardy plants and in part to the fact that plant physiology is further developed than

insect physiology, the study of cold resistance in plants has proceeded to a farther stage than the study of cold resistance in insects. From workers in plant physiology we can gain light on certain general physico-chemical problems involved in low temperature studies. The cause of death from low temperature has been considered, (1) as due to direct mechanical injury, (2) water loss through changes in permeability, and (3) precipitation of proteins or other irreversible chemical effect. The development of cold hardiness through slow growth, dehydration, or exposure to low temperatures showed that cold resistance is a dynamic rather than a static condition. Finally, the most recent work on plants has emphasized certain chemical and physical conditions of the plant tissues to be associated with cold resistance. These are low water content, efficient hydrophylic

colloids, and proteins not easily precipitated by low temperature.

The study of survival of insects at low temperatures has gone through (1) the observation stage, (2) the qualitative experimental, and is now entering the third stage, the quantitative. Studies on hibernation, on individual insect temperatures, and on heat regulation in insects, have all contributed to the general problem of freezing and survival of insects at low temperatures. Determinations for some insects have thus far been made as to the intensity of low temperatures they can survive, and whether or not they can withstand ice formation in their tissues. Aside from the finding that low water content is associated with cold resistance there yet remain to be discovered the physico-chemical factors associated with or causing cold hardiness in insects.

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BIOGRAPHY

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From 1918 to 1920 she was student assistant in entomology at Kansas State College. During 1920 and 1921 she was graduate assistant in zoology and entomology at the same institution. The following year she was instructor in mathematics and chemistry at Lindenwood College, St. Charles, Missouri. The summer of 1922 she served as preparator of specimens for comparative anatomy at Kansas State College. During the years 1922 to 1925 she has been an assistant in entomology and a graduate student at the University of Minnesota.

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